Comparative Study of WR 225448 and Primaquine in the *Plasmodium cynomolgi* - Rhesus Monkey Radical Curative Model, Phase II

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OBJECTIVES

1. To compare the efficacy of WR 225448-chloroquine with the radical curative combination of primaquine-chloroquine.

2. To compare the efficacy of WR 225448 alone with the combination WR 225448-chloroquine.

3. To determine whether WR 225448 is efficacious in a single oral dose.

BACKGROUND : In rhesus monkeys infected with sporozoites of *P. cynomolgi*, primaquine (in combination with chloroquine) is a radical curative drug. In this combination, primaquine cures most infections at a total dose of 3.5 mg. base per kg. body weight, whether that dose is a single dose or divided into 3 or 7 daily doses (1).

In man, the toxicity of primaquine precludes administration in a single curative dose. Thus, to achieve a radical cure of *P. vivax* in man, the dose is ordinarily given in divided doses over 14 to 21 days (in conjunction with a 3-day course of chloroquine).

WR 225448, an experimental 8-aminoquinoline, appeared to be as potent as primaquine as a tissue schizonticide and had exceptionally potent blood schizonticidal activity. The toxicity of WR 225448 in the rhesus is currently being investigated.

Because of the apparent blood schizonticidal activity of WR 225448, the ability to achieve a radical cure of sporozoite induced *P. cynomolgi* without simultaneous administration of a second drug such as chloroquine was considered a real possibility. Since preliminary testing of WR 225448

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indicated that it had a better therapeutic index than primaquine, it was decided to also test its efficacy by a single oral dose.

The dose ranges (Table 1) selected for this study were based on assumptions that the new lot of WR 225448 used in this study would have approximately the same potency as the succinate salt used in preliminary studies and that WR 225448 would cure at the same total dose whether it was given in a single dose or in seven divided doses.

METHODS : Rhesus monkeys were inoculated intravenously with 5-20 X 10^5 *P. cynomolgi* sporozoites produced in *Anopheles dirus* mosquitoes. Each monkey was then assigned, by random selection, to a particular drug-dose regimen. (See Table 1).

Administration of drug in each monkey was initiated on the day after its initial parasitemia reached 5000/cmm. (chloroquine was given beginning on this day in the appropriate groups). Parasitemia was determined in each monkey, by blood smears, three times prior to inoculation, daily from day six post inoculation until three days after parasitemia was suppressed to zero, then every other day through day 40 and twice weekly thereafter. During relapses or recrudescences of parasitemia counts were made daily.

Monkeys in which parasitemia were cleared by drug were monitored through day 40 at which time they were splenectomized and then monitored for an additional 30 days. If negative at the end of this period they were considered cured. Monkeys in which parasitemia was not cleared by drug were terminated on day 40.

Monkeys in which the parasitemia was cleared by drug but then reappeared before day 40 were treated with chloroquine phosphate, orally, 5 mg/kg, for seven days (whether or not chloroquine was included in the original regimen). If the parasitemia was cleared by the chloroquine splenectomy was performed 20 days after clearance and the monkey monitored an additional 30 days. Splenectomized monkeys in which parasitemia reappeared were treated with chloroquine phosphate, orally, 5 mg/kg. for 7 days. Those animals in which parasitemia was cleared by this chloroquine treatment were monitored for an additional 50 days.

Results of each drug regimen were categorized as Cure, Relapse, or Recrudescence based on the following definitions:

Cure: No reoccurrence of parasitemia following original treatment.

2. Relapse: Failure of the curative drug to clear all tissue parasites Confirmed by administration of chloroquine to all monkeys with recurrent parasitemia followed by a <u>temporary</u> clearance of the parasitemia.

3. Recrudescence: Failure of the curative drug to clear all blood parasites. Confirmed by administration of chloroquine to all monkeys with recurrent parasitemia followed by a <u>permanent</u> clearance of parasitemia.

RESULTS : Results for each treatment regimen are summarized in Table 2.

A combination of WR 225448 and chloroquine was clearly the most potent regimen. In combination with chloroquine, the minimum curative dose of WR 225448 was 0.875 mg. base/kg. body weight. Administered alone, WR 225448 was definitely curative at 7.0 mg. base/kg. body weight and one out of two monkeys was cured at 3.5 mg. base/kg. body weight. Contrary to Phase I, this study appears to indicate that WR 225448 has slightly more tissue schizonticidal activity in combination with chloroquine than alone.

Primaquine, in combination with chloroquine, cured at 7.0 mg. base/kg. body weight and at 3.5 mg. base/kg. body weight in one of two monkeys. As was determined in the Phase I study, WR 225448 is at least twice as potent as primaquine as a tissue schizonticide when administered alone in a single oral dose. WR 225448 proved to be at least 4 times as potent a tissue schizonticide as primaquine when administered in combination with chloroquine.

REFERENCE :

1. Brown, J.L., et. al., AFRIMS Annual Progress Report 1977-78, pp. 159-161.

Table I. Treatment Regimens

Dosage of WR225448 or Primaquine mg.Base/Kg.Body Weight	TREATMENT GROUPS						Dosage of Controls mg. Base/ Kg. Body Weight
	WR225448*	WR225448* & Chloroquine**	Primaquine* & Chloroquine**	CONTROLS			
				Primaquine*	Chloroquine**	Vehicle**	
14.0	2***		2				
7.0	2		2				
3.5	2		2				
1.75	2						
0.875	2	2					
0.4375	2	2					
				2			7.0
					2		3.1
						2	0

1. In all cases when Chloroquine is given in conjunction with another drug (WR225448 or Primaquine) the dose of Chloroquine was 3.1 mg. Base/Kg. Body Weight.

2. * Single oral dose
   ** Seven daily oral doses
   *** Number of monkeys/dose

Table II. Summary of Results.

Dosage of Primaquine mg./Kg. Body Weight	TREATMENT GROUPS						
	WR225448*	WR225448* & Chloroquine**	Primaquine* & Chloroquine**	CONTROLS			
						Vehicle**	
14.0	G400-Recrudescence G416-Cure		G409-Cure G413-Cure				
7.0	G426-Cure G429-Cure		G408-Cure G427-Cure				
3.5	G403-Cure G407-Recrudescence		G405-Cure G412-Recrudescence				
1.75	G415-Recrudescence G418-Recrudescence						
0.875	G402-Relapse G423-Recrudescence	G399-Cure G422-Cure					
0.4375	G406-Relapse G410-Relapse	G430-Relapse G434-Relapse					
				G419-Recrudescence G421-Recrudescence			7.0
					G411-Relapse G431-Relapse		3.1
						G414-No Effect G425-No Effect	0

Dosage of mg.Base/Kg. Body Weight

1. In all cases when Chloroquine is given in conjunction with another drug (WR225448 or Primaquine) the dose of Chloroquine was 3.1 mg.Base/Kg. Body Weight.
2. * Single oral dose
3. ** Seven daily oral dose